USM J/P-06

BAHAGIAN PENYELIDIKAN & PEMBANGUNAN
CANSELORI
UNIVERSITI SAINS MALAYSIA

Laporan Akhir Projek Penyelidikan Jangka Pendek

1) **Nama Penyelidik:** Dr Anil Dasgupta

Nama Penyelidik-Penyelidik Lain *(Jika berkaitan)* :

Dr Nicholas Jackson

Dr Norazmi Bin Mohd Nor

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3) **Tajuk Projek:** The Immunotype of Acute Leukaemia in Kelantan, as Determined by Flow Cytometry

4) (a) **Penemuan Projek/Abstrak**

*(Perlu disediakan makluman di antara 100 - 200 perkataan di dalam **Bahasa Malaysia dan Bahasa Inggeris** Ini kemudiannya akan dimuatkan ke dalam Laporan Tahunan Bahagian Penyelidikan & Pembangunan sebagai satu cara untuk menyampaikan dapatan projek tuan/puan kepada pihak Universiti).*

please see appendix A

appendix A

Incidence and cell surface immunophenotype of acute leukaemias in Kelantan

A Dasgupta

Abstract
with future direction of study

The present study reports the total number of acute leukaemias diagnosed in Kelantan over a period of 36 months by morphology, cytochemistry and flow cytometry or immunophenotype using ten monoclonal antibodies (to cell surface CD or HLA-DR antigens) coupled with fluorescein isothiocyanate (FITC) & phycoerythrin (PE).

A total of 69 cases gives an annual incidence of 1.4 per 100,000 population. Acute lymphoblastic Leukaemia (ALL) is more in children and acute myeloblastic leukaemia (AML) at all ages, but increases with age. The racial origin is roughly in proportion to the ethnic make up of Kelantan and the ratio between male and female is 1.1 : 1. In the older age group, the number of cases is higher in female and in contrast, the number of cases in the younger age group is higher in male.

Of these cases, about 50% expressed those antigens which are not associated with the main lineage and were diagnosed as biphenotypic which is higher in this study than those reported elsewhere. The expression of CD34 stem cell antigen with or without CD7 or the expression of CD7 with or without CD34 in AML was statistically significant. Whether the coexpression of antigens and an inverse relationship between AML & B-ALL shown in this study affect the prognosis of the disease remains to be ascertained suggesting that it requires further study with future direction as follows:

(a) the immunophenotype & karyotype or gene rearrangement, in addition to the traditional microscopy for cell morphology and cytochemistry of peripheral & bone marrow cells,

(b) the outcome of chemo or bone marrow therapy, and

(c) the long term follow up of cases.

Our preliminary data on survival rate is difficult to interpret because of short term follow up and small number of cases.

(b) **Senaraikan Kata Kunci yang digunakan di dalam abstrak:**

Bahasa Malaysia	**Bahasa Inggeris**
.....	.....
.....	.....
Leukaemia	.....
.....	Acute myeloblastic Leukaemia,
.....	Acute lymphoblastic Leukaemia
.....	Mixed lineage or biphenotypic
.....	CD antigens,
.....	HLA-DR antigen
.....	.....
.....	.....

5) **Output Dan Faedah Projek**

(a) Penerbitan *(termasuk laporan/kertas seminar)*
(Sila nyatakan jenis, tajuk, pengarang, tahun terbitan dan di mana telah diterbit/dibentangkan).

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teaching

(a) postgraduate MMed(path) students

(b) undergraduate Laboratory based rotation MD students

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publication (one more copy is enclosed herewith)

(a) Incidence and cell surface immunophenotype of acute leukaemias in Kelantan, Asia Pacific Journal of Molecular Biology and Biotechnology, Volume 3, No.2, June, 1995, pp 130-134

(b) Faedah-Faedah Lain Seperti Perkembangan Produk, Prospek Komersialisasi Dan Pendaftaran Paten.
(Jika ada dan jika perlu, sila gunakan kertas berasingan)

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(c) Latihan Gunatenaga Manusia

i) Pelajar Siswazah .teaching.to.....
(a) undergraduates
.....(b).postgraduates.....

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ii) Pelajar Prasiswazah: .....

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iii) Lain-Lain : .....presentation.....
(a) 1st National Conference
.....
in Haematology, 1994
.....
held in Kuala Lumpur,
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6. **Peralatan Yang Telah Dibeli:**

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UNTUK KEGUNAAN JAWATANKUASA PENYELIDIKAN UNIVERSITI

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untuk pengesahan &
tandatangan Dekan

DATO' PROFESOR MUSTAFFA EMBONG
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Incidence and Cell Surface Immunophenotype of Acute Leukaemias in Kelantan

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Abstract. The present study reports the total number of acute leukaemias diagnosed in Kelantan over a period of 16 months by morphology, cytochemistry and flow cytometry. A total of 38 cases gives an annual incidence of 1.3 per 100,000 population. Two cases could not be assigned a cell lineage. Of the remaining 36 cases, 39% were found to express the stem cell marker CD34 and 50% were biphenotypic which is higher than some studies reported elsewhere.

INTRODUCTION

Acute leukaemias are neoplasms resulting from the clonal expansion of haemopoietic blast cells, the exact cause of which is still unknown. The diagnosis of the disease has traditionally been confirmed by morphology of peripheral blood or bone marrow cells in conjunction with cytochemistry (1). Recently, immunophenotyping (2,3) and cytogenetics (4) have played increasingly important roles in the classification of these diseases (5). Some of the leukaemias may be confidently diagnosed from morphology of the peripheral blood or bone marrow but others require immunological or even molecular techniques to accurately assign a lineage and thus give appropriate treatment. The detection, in some cases, of antigens normally associated with different lineages on the same leukaemic cell has raised questions about whether such co-expression has any effect on prognosis or management (6). In this study, we have sought to establish the incidence and immunophenotypic spectrum of acute leukaemia in Kelantan.

MATERIALS AND METHODS

Study population. All cases of acute leukaemia which were diagnosed at the Hospitals of the Ministry of Health and the Universiti Sains Malaysia in Kelantan during the period from 1 st December 1992 to 31 st March 1994 were studied. The diagnosis of acute leukaemia was made by cytomorphology and cytochemical studies which were performed by Giemsa and enzymatic stain, respectively. Myeloblastic leukaemia cases were classified according to the French-American-British (FAB) classification (7). Cases were assigned as acute myeloblastic leukaemia (AML) if the cells were positive for either peroxidase or esterase. If these two stains were both negative, then the case was assigned as acute lymphoblastic leukaemia (ALL), unless the immunophenotyping results showed expression of

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pure myeloid antigen, in which case it was assigned as AML.

Cell surface immunophenotyping. A panel of directly conjugated fluorescent monoclonal antibodies (MoAb) were obtained from Becton Dickinson. Half the MoAb were labelled with fluorescein isothiocyanate (FITC) and the other half with phycoerythrin (PE) which emit green and red fluorescence respectively. They were used in the combinations as shown in Table 1. Specimens of peripheral blood or bone marrow in EDTA received by the laboratory were subjected to standard Ficoll-hypaque density gradient centrifugation. An aliquot of mononuclear cells thus isolated was stained by modified Giemsa to determine the percentage of blasts in the cell preparation to aid in the setting of light scatter gates. Details of cell preparation by density gradient, morphological evaluation by the different stains, and analysis of cell surface phenotype by the flow cytometer were those found in the laboratory manual (8) provided along with the FACScan by Becton Dickinson. For each MoAb, at least 10,000 mononuclear cells were analysed by the Flow Cytometer (FACscan) equipped with an argon-ion-laser operating at 488 nm wavelength. For analysis, the mononuclear cells were divided into groups based on forward and right angle light scatter characteristics (9) in which forward light scatter measures size, and right angle light scatter measures the complexity of the cells. In this way, the blast cell population was selected and a 2D dot plot was generated for each pair of MoAb. The percentage of cells positive for each marker was calculated by the subtraction computer analysis programme Lysys II (10).

Expression of an antigen on more than 30% of the blast cells was taken as positive. Co-expression of at least one lymphoid associated antigen with at least one myeloid-associated antigen on leukaemic blasts was considered as biphenotypic.

RESULTS

Incidence. Over the period of 16 months, 38 cases of acute leukaemia were diagnosed among the 1.6 million population of Kelantan which is one of the states of peninsular Malaysia, indicating that acute leukaemias occur at an annual rate of at least 1.8 per 100,000. The age/sex distribution is shown in Table 2. The age of the patients ranged from 8 days to 79 years, of which 21 were male and 17 female, the ratio being 1.2:1. Age-related incidence of acute leukaemia is also shown in Figure 1, indicating that AML occurs in all age groups whereas ALL peaks in childhood. The median age of the ALL cases was 5 as compared to 26 for AML. The racial origin of the patients was as follows: 35 Malays, 2 Chinese and 1 Indian, which is roughly in proportion to the ethnic make up of Kelantan.

Twenty two cases were ALL and 14 were AML. Two cases which could not be assigned a definite lineage by any of the means utilised in this study were called indeterminate acute

Table 1. The monoclonal antibodies (MoAb) for immunophenotyping were used in five pairs. In each pair, one MoAb was coupled to fluorescein isothiocyanate (FITC) and one to phycoerythrin (PE). These pairs are shown with their respective cell lineage associations.

FITC		PE	
CD10	(preB cells)	CD19	(B cells)
CD3	(T cells)	CD22	(B cells)
CD7	(T cells/NK cells)	CD33	(Myeloid cells)
CD34	(stem cells)	CD14	(Monocytes)
HLADR	(DR+ cells)	CD 13	(Myeloid cells)

Table 2. Age & sex distribution of acute myeloblastic leukaemia (AML), acute lymphoblastic leukaemia (ALL), and indeterminate acute leukaemia (IDL) over a period of 16 months.

Type of lineage	Age (years)	Males	Females	Total
AML	0-19	1	4	5
(n=14)	20-79	4	5	9
ALL	0-19	11	6	17
(n=22)	20-79	3	2	5
IDL	0-19	1	0	1
(n=2)	20-79	1	0	1
Total		21	17	38

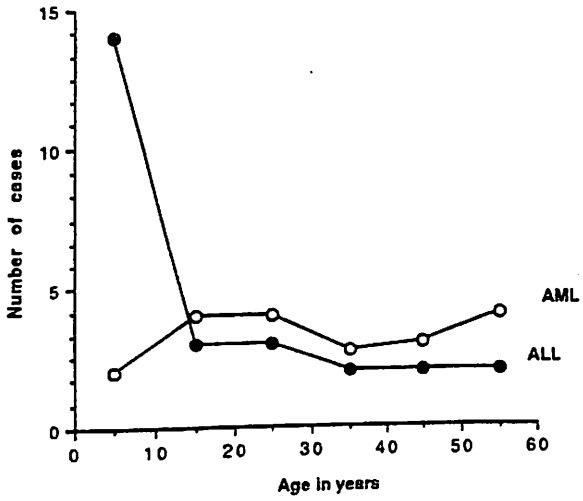

Figure 1. Age-related incidence of acute leukaemias in Kelantan.

leukaemia (IDL) and, are not part of the remainder of this study.

Immunophenotype. Table 3 shows the immunophenotype of the ALL and AML cases. CD13 and CD33 were expressed on the cell surface of 13 out of 14 patients with AML whereas CD19 and CD22 were expressed on the cells of all patients with B-lineage ALL, and CD3 and CD7 on all patients with T-lineage ALL.

Of the 36 cases, 39% expressed CD34 (Table 4), this being less common in patients with AML (29%) than in those with ALL (45%). The CD34 expression was higher in the biphenotypic cases (50%) than in the non-biphenotypic (32%) but this difference was not statistically significant.

Biphenotypic expression. The number of cases expressing the different antigens is shown in Table 3. Of the 36 AML and ALL cases, 17 cases expressed one or more CD antigens which are not normally associated with the main assigned lineage. 5/14 AML cases expressed lymphoid antigens and, among the ALL cases, 11/20 B-lineage and 1/2 T-lineage cases expressed myeloid antigens. As CD7 is not strictly T-cell restricted, and CD7+ AML is well recognized, the CD7+ AML cases were not called biphenotypic. Thus, altogether 14 cases (39%) were biphenotypic. In the 12 biphenotypic ALL cases, the number of blasts expressing myeloid antigens varied from 47.4% to 91.25%. In the two cases of biphenotypic AML, the number of blasts expressing lymphoid antigens was 58.4% and 77.27%, in the CD3+ and CD19+ cases respectively.

Table 3. The CD antigen and HLA-DR expression of the 14 cases of AML and 22 cases of ALL in this study.

	Cell surface antigens									
Lineage association	B			T		myeloid			stem cell	
CD	10	19	22	(7)	3	13	33	14	34	HLA-DR
AML (n=14))		1		3	1	13	13	2	8	8
ALL (n=22)										
B lineage (n=20)	16	20	20			11	3	2	8	20
T lineage (n=2)				2	2	1		1		2

Table 4. The expression of CD34 by the 14 cases of AML and 22 cases of ALL according to whether they were biphenotypic or not.

	CD 34+ positive	CD34- negative	%positivity
AML (n=14)	4	10	29
biphenotypic	1	1	50
non-biphenotypic	3	9	25
ALL (n=22)	10	12	45
biphenotypic	6	6	50
non-biphenotypic	4	6	40
Total (n=36)	14	22	39
biphenotypic	7	7	50
non-biphenotypic	7	15	15

DISCUSSION

The annual incidence of acute leukaemia in Kelantan (1.8 per 100,000) and the male: female ratio (1.2: 1) as obtained from this study are similar to Western figures of 2-4 per 100,000 and 1.3 respectively (11). As in the West, AML occurs in all ages, whereas ALL peaks in the first two decades of life. Eighty percent of patients with B-lineage ALL were CD10+ but these cases could not be categorized into the 'common ALL' and 'pre-B ALL' because cytoplasmic mu staining was not performed. Expression of CD14 was found in only 2 cases of AML and, disappointingly for a monocytic marker, there was no correlation with the FAB subtype (data not shown).

In spite of the fact that our knowledge on lineage specificity has increased with the advent of immunophenotyping, the specificity of monoclonal antibodies (12) is still being evaluated by studies on normal lymphoid tissues and bone marrow (13). However, expression of CD19, CD20 or CD22 on the cell surface are taken as B cell markers; CD3 as a T cell marker and CD13, CD33 or CD14 as myeloid cell markers to assign a lineage. In view of this, the positivity of CD19 or CD3 in patients with AML and CD13, CD33 or CD14 in ALL was taken as the basis of biphenotype, the percentage of which appeared to be higher in this study (50%) than most studies published in the West which vary from O to 50% (13). Whether such a widely variable incidence is due to (a) the application of variable diagnostic criteria, (b) the difficulty in dissociating leukaemic blasts from normal cells that may pose problems in establishing accurate electronic gates around leukaemic blast cells, (c) the subjectiveness of cell surface immunophenotypic interpretation, or (d) the lack of standardized technique, is still being debated (6). It has also been debated whether such biphenotypic acute leukaemias have a worse clinical prognosis as compared to non-biphenotypic cases (14) but a consensus on this issue has yet to be reached. As quite a high proportion of our patients either refuse treatment at the outset or default from treatment, we have not been able to analyse whether those with biphenotypic leukaemia have a worse prognosis.

Although we found a higher rate of expression of CD34 amongst biphenotypic than non-biphenotypic leukaemias, the difference was not statistically significant. It has been reported elsewhere (15) that biphenotypic leukaemias have an increased expression of CD34. As CD34 is expressed on stem cells, this has been taken to imply that biphenotypic leukaemias arise from such early progenitor cells but this remains controversial.

In conclusion, this study shows that acute leukaemia is at least as common in Kelantan in peninsular Malaysia as in other parts of the world. The proportion of cases which are biphenotypic appears higher than elsewhere but the significance of this finding requires further study.

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